DYNAMICS OF LEAF AND FINE ROOT LITTER DECAY IN TEMPERATE FORESTS

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by

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ABSTRACT

Leaf and fine root litter decomposition is central to biogeochemical cycles in northern forest. I studied the nitrogen translocation to leaf litter during decomposition, and the fine root decomposition process. Nitrogen immobilization in fresh litter represents a significant N flux in forest ecosystems, yet its sources, controls, and implications are not well studied. I conducted two leaf decay experiments, using ^{15}N-labeled sugar maple leaf litter, to quantify N transport from old litter and soil to fresh litter during early stages of decomposition, and I examined the influence of litter N concentration and soil N availability on upward N transfer in a northern hardwood forest. After one year of decay, the average N transfer from soil to fresh litter (2.63 mg N g^{-1} litter) was much higher than the N transfer from older litter (1 to 2 yr old) to fresh litter (0.37 mg N g^{-1} litter). As an indicator of N transfer efficiency from these N sources into decaying litter, we calculated the ratio of annual N transfer / excess ^{15}N pool for these two N sources. The ratio was not significantly different between old litter and soil, suggesting that fungi utilize N in the old litter and mineral soil pools for transport to decaying fresh litter with similar effectiveness. Litter N concentration had a significant effect on upward N flux into decaying leaf litter, whereas no effect of soil N fertilization was observed. These results illustrate the mechanisms whereby continuing N deposition will affect the important process of N translocation into decaying litter. Future work characterizing the fungal taxa involved in this process and their responses to changing environments is needed. Another of my studies is on fine root decomposition. Despite its importance in global biogeochemical cycling, fine root decomposition has received limited attention, and factors that regulate this process are not well

understood. Most studies on fine root decomposition have been based on litterbag experiments, but the appropriateness of this approach has been questioned. In this study, I compared fine root decay using litterbag and intact core approaches, and I evaluated the role of fungal hyphal networks, root chemistry, and soil environment in regulating root decay rates and decomposer communities. I used 454 pyrosequencing to survey and compare the fungal communities on decaying fine roots from different samples. The results show that 1) fine roots decaying in intact cores have a significantly higher rate of mass loss and N, P release than fine roots decaying in litterbags; 2) there is an obvious difference between fungal communities developed on intact core root samples and those on litterbag root samples; 3) by rotating intact cores and disrupting the formation of fungal hyphal networks, fungal decomposer communities on fine roots were altered, yet the decay rates of fine roots did not change and; 4) root chemistry, rather than soil environment, has a significant effect on fine root decomposition rate. NCBI BLAST search of the most abundant fungal operational taxonomic units in this study found ectomycorrhizal fungi orders such as Boletales, Thelephorales, and Cantharellales on roots after half year and one year of decay, suggesting that ectomycorrhizal fungi may become saprotrophic after root senescence. Further phylogenetic analysis is needed to identify the important fungal species that are involved in fine root decomposition, which will shed further light on the controls of the fine root decay process.

BIOGRAPHICAL SKETCH

As the son of a high school history teacher and a college physics lecturer, Ang Li grew up in a family of educators in the city of Tianjin, China. With some luck and effort, he entered Nankai High School when he was twelve years old, where two of China's prime ministers had graduated. Six years of high school life turned him into a quiet boy, able to sit and study for hours. Upon graduation from high school, Ang enrolled in Peking University in Beijing, China. He graduated with B.S. in ecology and statistics after four years of hard study, and came to Cornell University to study forest ecology, following Tim Fahey. He conducted research on leaf and fine root litter decomposition. Later he rediscovered his interest on quantitative subjects. From the fall of 2012, he will go to the University of Chicago to pursue PhD study in statistics.

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I am greatly thankful for the advice and support I received from my another advisor Teresa Pawlowska. Previously I had no background or experience in biochemistry and molecular biology, it is under her guidance that I gradually learned some important molecular biology experiments, and applied them in my research. She was very generous to me on the access to her lab resources, very patient when explaining to me the different concepts and important issues in experiments, and helped me overcome the troubles I met. I benefitted immensely from the discussions with her and her lab members. Besides, I also received the encouragement from

Teresa many times, to take the challenge and think of ways to improve my work. From the bottom of my heart, I appreciate the help that Teresa has provided to me.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

NITROGEN TRANSLOCATION TO FRESH LITTER IN NORTHERN HARDWOOD FOREST

ABSTRACT:

Nitrogen immobilization in fresh litter represents a significant N flux in forest ecosystems, yet its sources, controls, and implications are not well studied. We conducted two leaf decay experiments, using ^{15}N-labeled sugar maple leaf litter, to quantify N transport from old litter and soil to fresh litter during early stages of decomposition, and we examined the influence of litter N concentration and soil N availability on upward N transfer in a northern hardwood forest. After one year of decay, the average N transfer from soil to fresh litter (2.63 mg N g^{-1} litter) was much higher than the N transfer from older litter (1 to 2 yr old) to fresh litter (0.37 mg N g^{-1} litter). As an indicator of N transfer efficiency from these N sources into decaying litter, we calculated the ratio of annual N transfer / excess ^{15}N pool for these two N sources. The ratio was not significantly different between old litter and soil, suggesting that fungi utilize N in the old litter and mineral soil pools for transport to decaying fresh litter with similar effectiveness. Litter N concentration had a significant effect on upward N flux into decaying leaf litter, whereas no effect of soil N fertilization was observed. These results illustrate the mechanisms whereby continuing N deposition will affect the important process of N translocation into decaying litter. Future work characterizing the fungal taxa involved in this process and their responses to

changing environments is needed.

INTRODUCTION:

Nitrogen is strongly immobilized during early stages of litter decay in forests (Melillo and others 1982; Blair and others 1992; Parton and others 2007), and fungal translocation from underlying soil is a primary mechanism contributing to N accumulation in decaying litter; indeed, fungi may be responsible for more than 90% of total upward N transport into fresh litter (Frey and others 2000). Many saprotrophic fungi form extensive hyphal networks that are capable of rapid translocation of carbohydrates, mineral nutrients, and water within their mycelia (Boddy 1999; Tlalka and others 2002, 2003, 2008), which enables them to overcome local resource limitations and colonize heterogeneous substrates (Boberg and others 2009). Therefore, to proliferate on C-rich, N-poor leaf litter, fungi translocate soil inorganic N to surface residues, a process that is one of the largest N flux pathways in forests (Hart and Firestone 1991). Moreover, leaching of N from fresh litter occurs coincidentally with fungal translocation into litter so that the gross N flux is even greater than the net flux (Berg and Staaf 1981). For example, in a tracer study with ^{15}N-labeled sugar maple litter, Fahey and others (2011) observed a very high gross N translocation into fresh litter during the first year of decomposition, equivalent to 60% of initial litter N content. This N flux would naturally derive mostly from old litter and soil, yet the relative contribution from these two sources is still unknown.

In a meta-analysis of global litter decomposition patterns, Parton and others (2007) found that

net N immobilization of leaf litter is primarily determined by the initial tissue N concentration, suggesting that litter chemistry should have a strong influence on the rate of fungal N translocation to fresh litter. However, in a laboratory experiment, Frey and others (2000) found that soil inorganic N availability had a more consistent effect on fungal N translocation than substrate quality. Thus, whether litter chemistry or soil N availability is the dominant control on soil-to-litter N translocation is not entirely clear.

From a functional view, soil N translocation to fresh litter allows fungi and other microbial decomposers to proliferate on fresh litter and build extracellular enzymes, thereby facilitating early decomposition and possibly shortening the time to initiate net N mineralization from decaying litter. Moreover, this upward N flux mobilizes N from deeper soil horizons, with possible implications for N availability to plants and for soil organic N and C stabilization. Despite its importance, soil-to-litter N translocation has received limited attention. The aim of the present study was to quantify N transport from old litter and soil to fresh litter during early decomposition, and to examine the influence of litter chemistry and soil N availability on this process. We hypothesized that 1. N would be translocated to fresh litter more effectively from old litter than from underlying soil because of the proximity of the litter; and 2. Fresh litter N concentration would have a greater effect on N translocation to litter than soil N availability, following the previous observation of Parton and others (2007). We hoped that results from this study would provide a better understanding of the internal N cycling of forest soil, relevant for predicting ecosystem responses to anthropogenic N loading (Aber and others 1998).

METHODS:

Study area and field experiments:

To examine the gross transport of N to decaying leaf litter, we employed ^{15}N as a tracer in two separate experiments. Experiment 1 was designed to compare the magnitude of N transport to fresh leaf litter from old litter (1-2 year old litter) and from underlying soil, testing hypothesis 1. Experiment 2 was designed to compare the effects of litter N concentration compared to soil N availability in regulating N transport to decaying litter, testing hypothesis 2. Both experiments were conducted in a sugar maple stand in Cornell University's Arnot Forest, Tompkins County, NY (42°15'N, 76°40'W). Mean temperature is -4°C in January and 22°C in July, and mean annual precipitation is 90 cm, evenly distributed through the year. The stand is a ca. 70-yr-old post-agricultural forest with thin (2 cm) forest floor. Soils are acidic Dystrochrepts (pH 4.5-5.0) (Fahey and others, 2011).

For experiment 1 we collected fresh unlabeled sugar maple leaf litter from the study site during fall 2010. Litter was air-dried before preparation of litterbags. A total of 36 litterbags (30 × 30 cm) were constructed from nylon-coated plastic window screen (1 mm mesh size) and 27 g dry weight of unlabeled litter was added to each bag, representative of the roughly 300 g m^{-2} per year leaf litterfall in the study area (Fisk and others 2004). Litterbags were deployed in three treatments: 1) control, 2) ^{15}N labeled "old litter" and 3) ^{15}N labeled soil. The control plots and "old litter" plots were located randomly in the forest stand, and litterbags were positioned

directly on the surface of the forest floor on Dec 13th, 2010. In the "old litter" plots, surface Oi litter was removed from 50 × 50 cm plots and replaced with partially-decayed leaf litter (labeled with ^{15}N), collected from another experiment (Fahey and others 2011). Specifically, we added a total of 194 g m^{-2} of one-year-old litter ($\delta^{15}N$ = 1150 ‰, [N] = 2.2 %) and 117 g m^{-2} of two-year-old litter ($\delta^{15}N$ = 819 ‰, [N] = 2.4 %), to represent the corresponding quantities remaining of 300 g m^{-2} per year leaf litterfall (Fahey and others 2011). Litterbags with unlabeled litter ($\delta^{15}N$ = -1.7 ‰, [N] = 1.1 %) were then placed on top of this ^{15}N labeled old litter. The third set of plots (^{15}N labeled soil) were located 50 m away from the control and old litter plots. These plots were watered with aqueous solution of 99 atom % enriched $^{15}NH_4Cl$ in May 2006 to deliver tracer quantities of ^{15}N (0.25 g N m^{-2}). Prior to the initiation of this experiment, all fresh leaf litter was harvested from these plots each autumn thereafter and replaced with roughly equal amount of unlabeled litter; hence the ^{15}N enrichment was in underlying soil. Litterbags were placed directly on the forest floor of these plots. We estimated the pool size of ^{15}N in the upper 10 cm of soil in this treatment based on analysis of total ^{15}N in soil samples collected from the site in summer 2010 and soil bulk density measurements from a nearby stand (Fahey and others 2011).

For experiment 2 we established 24 plots in the same mature sugar maple stand as experiment 1. Twelve randomly chosen plots were fertilized monthly from September to November 2010 with 1 g N m^{-2} NH_4NO_3 (in aqueous solution each time), and 12 plots were irrigated controls. This procedure increased available N (KCl-extractable inorganic N, including NH_4 and NO_3) by 21%

in forest floor, and by 53% in upper mineral soil. Two sets of litterbags containing ^{15}N labeled sugar maple leaf litter were prepared using litter samples from the labeling chambers (Fahey and others 2011). One set of litterbags contained early-season litter (collected in Sept & Oct, 2010) of high N concentration ($\delta^{15}N$ = 485 ‰, [N] = 1.3 %) and the other set of litterbags contained late-season litter (collected in Nov, 2010) of low N concentration ($\delta^{15}N$ = 493 ‰, [N] = 0.8 %). Litterbags were prepared as for experiment 1 and half of each set was placed randomly on the fertilized and control plots, on Dec 12th, 2010. For both experiments, subsamples of litterbags were retained for chemical analysis, described below.

Half of the litterbags were collected randomly for each experiment on May 26th, 2011 and Oct 31st, 2011. Samples were returned to the laboratory and dried to constant weight at 70°C. Samples were weighed to ± 0.001 g and ground to fine powder for chemical analysis. Samples were analyzed for C, N, and ^{15}N, at Cornell University Stable Isotope Laboratory. As an indicator of the analytic precision, the coefficient of variation for C %, N %, and $\delta^{15}N$ measurement of ground cabbage standard sample (BSSL-100, ^{15}N enriched sample) were 0.64%, 0.93%, 0.37%, respectively.

Data Analysis and Statistics:

We calculated the gross N transport to decomposing fresh litter from old litter and soil on the basis of changes in dry mass and atom % ^{15}N concentration for each litterbag sample. Because N and ^{15}N are coincidentally transported into and out of the litter during decomposition (Berg

and Staaf 1981), to calculate the gross N transport to the litter from soil we must account for both processes (Fahey and others 2011). For experiment 1 (except the control), the gross amount of N transported from ^{15}N labeled old litter into decomposing fresh leaf litter was calculated with the mass balance equation:

Change of ^{15}N mass of decomposing leaves = ^{15}N upward translocation into decomposing leaves – Loss of leaf ^{15}N during decomposition and leaching

In which:

^{15}N upward translocation into decomposing leaves = $\underline{\text{N upward translocation}} \times {}^{15}N/{}^{14}N$ of old ^{15}N-labeled litter,

Loss of leaf ^{15}N during decomposition and leaching = Gross leaf N loss × average $^{15}N/^{14}N$ of leaf litter during decomposition, and

Gross leaf N Loss = Net leaf N loss + $\underline{\text{N upward translocation}}$

Net leaf N loss is the observed change of N content in decaying leaves. The average $^{15}N/^{14}N$ of leaf litter during decay was estimated as the average of initial and final $^{15}N/^{14}N$ value of decaying leaf litter. The amount of N translocation was calculated by solving the linear mass balance equation. The amount of N translocation from labeled mineral soil into decomposing fresh leaves was calculated in the same way. N transport into decomposing fresh leaf litter from old litter and mineral soil were compared by independent sample T test. Mass remaining, %N, % N remaining, and δ ^{15}N of leaf litter collected on May 26th and Oct 31st 2011, were compared among different treatments in experiment 1 using one-way ANOVA (Table 1.1).

For experiment 2, the upward N translocation from both old litter and mineral soil into fresh leaves was calculated with the equation:

N upward translocation = Current leaf N content – Original leaf N remaining in leaf litter

In which:

Original leaf N remaining = Initial leaf N mass × % ^{15}N remaining, and

where % ^{15}N remaining is the percentage mass remaining of ^{15}N in leaf litter. Since % ^{15}N remaining represents the percentage of original leaf N that is retained in leaf litter, the product of initial leaf N mass and % ^{15}N remaining is the amount of original leaf N that is retained. Differences in upward N transport to decomposing fresh leaf litter among litter types (early and late season fresh litters), treatments (fertilized and unfertilized plots), and sample time were analyzed by a mixed level (2×2×2) three-way factorial ANOVA. All statistical analyses were performed using SPSS software (2001, ver. 13.0, SPSS Inc., USA).

RESULTS:

Experiment 1. During the first sampling interval (Dec 12th, 2010 – May 26th, 2011), dry weight loss was slightly higher for litterbags in the control and "old litter" plots (26.2%) than the ^{15}N labeled soil plots (20.6%). By Oct 31st, 2011, dry weight loss decreased in the order: control > "old litter" > "labeled soil" (Table 1.1). Although N concentration increased steadily during litter decay in all treatments, net loss of N was observed, with an average 87% of original N remaining at the final collection.

Table 1.1 Average mass remaining, N concentration and N content remaining, and ^{15}N abundance of leaf samples from different treatments of experiment one, at Dec 12th, 2010, May 26th , 2011, and Oct 31st, 2011 (see text for explanation). Standard deviations are in the brackets following average values. Different superscript letters within dates indicate significant ($P < 0.05$) differences of mass remaining, N%, N remaining and $\delta\ ^{15}N$ among leaf samples from different treatments, at May 26th and Oct 31st, 2011, according to the results of One-way ANOVA with Tukey's HSD test.

Date	Treatment	Original mass remaining (%)	N (% dry weight)	Original N remaining (%)	$\delta\ ^{15}N$ (per mil)
Dec 12th, 2010	All	100%	1.10	100%	-1.72
May 26th, 2011	Control	73.18 ^{a} (0.63)	1.40 ^{a} (0.09)	93.12 ^{a} (5.62)	-1.66 ^{a} (0.64)
	^{15}N-enriched old litter	74.25 ^{a} (2.84)	1.38 ^{ab} (0.10)	93.20 ^{a} (3.97)	22.29 ^{b} (5.27)
	^{15}N-enriched mineral soil	79.43 ^{b} (4.24)	1.25 ^{b} (0.10)	90.00 ^{a} (4.95)	22.46 ^{b} (10.49)
Oct 31st, 2011	Control	51.17 ^{a} (1.70)	1.94 ^{a} (0.06)	90.22 ^{a} (3.90)	-1.28 ^{a} (0.24)
	^{15}N-enriched old litter	56.51 ^{b} (1.38)	1.80 ^{a} (0.10)	92.64 ^{a} (7.35)	38.68 ^{b} (10.35)
	^{15}N-enriched mineral soil	60.23 ^{b} (4.66)	1.45 ^{b} (0.11)	79.09 ^{b} (5.87)	71.28 ^{c} (18.76)

In the control plots, $\delta^{15}N$ remained nearly constant whereas $\delta^{15}N$ increased markedly in both treatments, indicating upward flux of ^{15}N from underlying litter and soil (Table 1.1). On the final date, $\delta^{15}N$ in litter was significantly higher in the ^{15}N labeled-soil than the old litter plots ($P = 0.004$; Table 1.1).

We estimated the gross N flux into decaying litter in experiment 1. The flux was significantly greater in the ^{15}N labeled soil than the old litter plots for both collection dates ($P = 0.064$ for May 26th collection, $P = 0.000$ for Oct 31st collection; Table 1.2). As a percentage of the initial litter N content, this gross N flux after one year of decay averaged 3.4% for the old litter and 23.9% for the ^{15}N-labeled soil plots.

Experiment 2. For both decay intervals, weight loss of ^{15}N labeled litter was similar to that of unlabeled litter in experiment 1 and did not differ significantly either between early and late season litter or between fertilized and irrigated control plots (Figure 1.1).

N concentration of early and late season fresh litter in both fertilized and irrigated control plots increased steadily during one year of decomposition. While net gain of N was observed in late season (low initial N) fresh litter, net loss of N was observed in early season (high initial N) fresh litter. At the end of the first year, early season fresh litter lost 21.6% total N, and late season fresh litter gained about 12.3% total N (Figure 1.1).

For both early season and late season fresh litters, $\delta^{15}N$ decreased markedly in both fertilized and irrigated control plots, presumably due in part to the dilution effect of upward flux of unlabeled N from underlying litter and soil. In one year of decomposition, ^{15}N content in early season

Table 1.2 Average gross N flux into decaying sugar maple leaf litter in experiment 1 and 2 on two collection dates. Standard deviations are in parentheses. For Exp 1, N flux into litter on ^{15}N labeled old litter plots accounts only for the N flux from old litter to fresh leaf litter, and N flux into litter on ^{15}N labeled soil plots accounts only for the N flux from soil to leaf litter. For Exp 2, N flux refers to the total upward N translocation into decomposing litter. Standard deviations are in the brackets following average values. Different superscript letters indicate significant ($P < 0.1$) differences of N flux, N flux / Initial litter N %, among leaf samples from different treatments in experiment 1, according to the results of independent sample T test, and among leaf samples from treatments in experiment 2, according to the results of one-way ANOVA with Tukey's HSD test.

Treatments	May 26th, 2011 collection		Oct 31st, 2011 collection	
	N flux (mg / g litter)	N flux/Initial litter N (%)	N flux (mg / g litter)	N flux/Initial litter N (%)
Experiment 1				
^{15}N-enriched old litter plots	0.18 [a] (0.07)	1.62 [a] (0.61)	0.37 [a] (0.14)	3.39 [a] (1.28)
^{15}N-enriched soil plots	0.71 [b] (0.62)	6.46 [b] (5.65)	2.63 [b] (0.55)	23.90 [b] (5.00)
Experiment 2				
Early season litter on fertilized plots	0.18 [a] (0.08)	1.41 [ab] (0.59)	1.06 [a] (0.29)	8.15 [a] (2.21)
Early season litter on unfertilized plots	-0.02 [a] (0.49)	-0.16 [b] (3.78)	1.18 [a] (0.11)	9.07 [a] (0.82)
Late season litter on fertilized plots	0.53 [a] (0.18)	6.66 [a] (2.30)	1.68 [b] (0.14)	21.02 [b] (1.73)
Late season litter on unfertilized plots	0.57 [a] (0.29)	7.11 [a] (3.67)	1.70 [b] (0.09)	21.27 [b] (1.10)

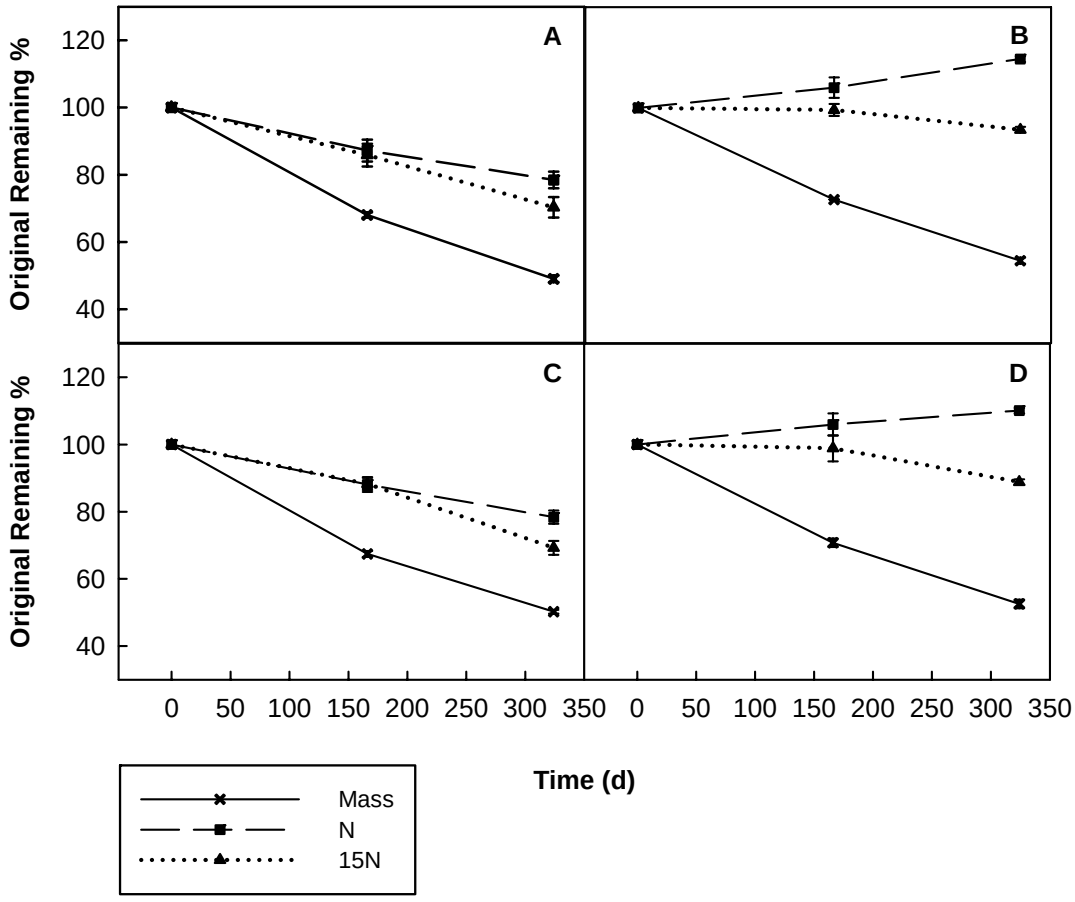

Figure 1.1 Percent of original mass, N, and ^{15}N remaining during litter decomposition in different treatments of experiment two: (A) early season fresh litter decomposing on fertilized plots, (B) late season fresh litter decomposing on fertilized plots, (C) early season fresh litter decomposing on unfertilized plots, (D) late season fresh litter decomposing on unfertilized plots. Error bars indicate standard errors.

fresh litter decreased faster than that in late season fresh litter ($P = 0.000$ by comparison of % ^{15}N content lost; Figure 1.1).

There was a significant effect of litter type, but no effect of fertilization treatment on the upward N transfer into fresh litter after one year of decomposition (Table 1. 3). N translocation into 1 g of late season fresh litter was much greater than the N translocation into 1 g early season fresh litter. In one year's decomposition, the average N translocation into late season fresh litter represented 21.2% of initial total N, and the average N translocation into early season fresh litter represented 8.6% of initial total N (Table 1.2). There was no significant difference in the amount of N transferred into fresh litters between fertilized and unfertilized plots, for both late season ($P = 0.844$) and early season fresh litter ($P = 0.557$).

DISCUSSION:

We quantified for the first time the separate contributions of old litter and mineral soil to the flux of N into decomposing leaf litter. Contrary to our expectation that more N would be translocated from old litter than from mineral soil over the first year of decay (hypothesis 1), we observed that gross N flux to decaying litter was much greater for mineral soil (2.63 mg N g^{-1} litter) than for 1 to 2 yr old litter (0.37 mg N g^{-1} litter). This result was surprising because leaf litter was immediately adjacent to the old litter ^{15}N source; however, the magnitude of the ^{15}N source differed between these two treatments. That is, the excess ^{15}N pool in the old litter (25.5 mg m^{-2}) was much smaller (on an area basis) than that in the upper 10 cm of soil (265.9 mg m^{-2}).

Table 1.3 Results of mixed-level three-way factorial ANOVA on the upward N transfer into fresh sugar maple leaf litter in a northern hardwood forest in central New York in experiment 2. The study compared upward N flux for high and low N litter ("litter type") and soil N addition ("fertilization") on two collection dates.

ANOVA			
Source of variation	df	P values	F values
Litter type	1	**0.000**	**26.661**
Fertilization treatment	1	0.944	0.005
Sample time	1	**0.000**	**116.593**
Litter type × Fertilization treatment	1	0.732	0.122
Litter type × Sample time	1	0.617	0.260
Fertilization treatment × Sample time	1	0.458	0.578
Litter type × Fertilization treatment × Sample time	1	0.414	0.704

Hence, as an indicator of fungal N transfer efficiency from N sources into decaying litters, we calculated the ratio of annual N transfer / excess ^{15}N pool in N sources. The average ratio for old litter and for mineral soil were 4.39 and 3.91, respectively, and not significantly different from each other ($P = 0.688$). Thus, fungi appear to utilize N in the old litter and mineral soil pools for transport to decaying fresh litter with similar effectiveness. By comparison, Fahey and others (2011) calculated that transport from one-year-old litter supplied about 18% of the gross N transport to decaying sugar maple litter.

Ideally the comparison of the effectiveness of N scavenging from old litter and soil might be calculated on the basis of net or gross ^{15}N mineralization. However, the mechanism whereby soil N is scavenged and transported by fungi to N-poor substrates is not well understood (Watkinson and others 2007). Lindahl and others (2002) proposed that fungal mycelium itself is the principal pool of N storage that is mobilized for this purpose. The transport involves more than just tissue growth into substrates (Watkinson and others 2007) and amino acid transport through fungal mycelium probably contributes to the large fluxes involved (Tlalka and others 2002, 2003). Although the exact mechanisms of fungal scavenging and transport require more study, our results indicate that the process is probably roughly equally active in surface organic horizons and upper mineral horizons in temperate forest soils.

Summing the N flux from mineral soil and old litter into decaying litter, we estimated a total annual N upward translocation into decaying litter at 0.90 g m^{-2}. This is very similar to the estimate of annual N translocation from mineral soil to forest floor in a California pine forest

(Hart and Firestone 1991) of 0.9 g m^{-2}, but higher than for a pine forest in southeastern Wyoming (Fahey and others 1985) of 0.4 g m^{-2}. In a laboratory ^{15}N soil labeling experiment, Frey and others (2000) calculated a total annual fungal mediated N flux of 2.4 g m^{-2}, which is considerably higher than our estimate. Differences between the experimental systems and especially the duration of the experiments may help to account for this observation: the experiment of Frey and others (2000) lasted only for 30 days, while ours lasted for nearly a year.

Frey and others (2000) suggested that both litter quality and N availability in soil are important controls on the rate of soil-to-residue N translocation. We found that litter N status had a significant effect on the N flux from old litter and soil into decomposing litter. Late season fresh litter, with a much lower N concentration, received significantly more upward N transfer than early season fresh litter (Figure 1.1, Table 1.2). In contrast, no effect of soil N fertilization was observed on upward N translocation (Figure 1.1; Table 1.2, 1.3). These observations support our second hypothesis that the process of N transport to litter is controlled primarily by the substrate chemistry (Parton and others 2007).

The magnitude of N flux into the fresh litter was somewhat lower in the present study than has been observed in other studies of litter decay in temperate hardwood forests (Gosz and others 1973; McClaugherty and others 1985). For example, we estimated that for the late-season (low N) litter N accumulation amounted to 12.3% of the original N content after one year of decay, whereas in a nearby sugar maple forest Fahey and others (2011) observed 26–32% for this N accumulation. Several differences could contribute to this lower flux in the present study.

First, the field incubations did not begin until December which might have reduced fungal colonization and N immobilization compared to mid-autumn. Second, in comparison with the Fahey and others (2011) and Gosz and others (1973) study sites, which were primary forest with deeper forest floor, the present post-agricultural forest had thin forest floor, and possibly less well-developed fungal networks. Also, litter N concentration in the present study (1.1 to 1.3%) was slightly higher than in the other studies.

Our results have implications for understanding the effect of N deposition on forest ecosystems. N saturation, the phenomenon that overabundance of N in forest soil leads to excessive nitrate leaching and soil acidification, has been extensively documented (Aber and others 1998; Aber and others 2003). However, despite long-term, moderately high N deposition, many forests in northeastern US are not N saturated (Martin and others, 2000; Goodale and others, 2003). The interactions of C and N in the surface organic horizon of cold temperate forests probably play a key role in this surprising behavior (Currie and others 1999). One possible reason is the high capacity for these forests to retain N in soil organic matter (Fahey and others 2011). Under high N deposition, a higher proportion of N demand in decaying leaf litter can be supplied from atmospheric N, which could reduce the upward translocation of N from older litter and mineral soil by fungi, and contribute to N stabilization in SOM.

Nitrogen transport to decaying fresh leaf litter is a major N flux in forest ecosystems in northeastern US. To better understand the mechanism of upward N transport in forest soil, future studies are needed to examine the influence of this N flux on litter decomposition (Frey

and others 2003), and its response to high N deposition. Moreover, detailed studies are needed to identify and better characterize the principal fungal taxa that translocate N from older litter and mineral soil to freshly decaying leaves. The same or related taxa of Basidiomycetes as are involved in wood decay are likely candidates (Watkinson and others 2007). Finally, measurements of fungal N transport efficiency from older litter and mineral soil and the physiology of N scavenging and transport are needed to understand likely implications of continuing N deposition on forest health and dynamics.

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CHAPTER 2

FINE ROOT DECOMPOSITION IN ORGANIC AND MINERAL SOIL OF AN ADIRONDACK PINE FOREST

ABSTRACT:

Despite its importance in global biogeochemical cycling, fine root decomposition has received limited attention, and factors that regulate this process are not well understood. Most studies on fine root decomposition have been based on litterbag experiments, but the appropriateness of this approach has been questioned. In this study, we compared fine root decay using litterbag and intact core approaches, and we evaluated the role of fungal hyphal networks, root chemistry, and soil environment in regulating root decay rates and decomposer communities. We used 454 pyrosequencing to survey and compare the fungal communities on decaying fine roots from different samples. Our results show that 1) fine roots decaying in intact cores have a significantly higher rate of mass loss and N, P release than fine roots decaying in litterbags; 2) there is an obvious difference between fungal communities developed on intact core root samples and those on litterbag root samples; 3) by rotating intact cores and disrupting the formation of fungal hyphal networks, fungal decomposer communities on fine roots were altered, yet the decay rates of fine roots did not change and; 4) root chemistry, rather than soil environment, has a significant effect on fine root decomposition rate. NCBI BLAST search of the most abundant fungal operational taxonomic units in this study found ectomycorrhizal fungi orders such as

Boletales, Thelephorales, and Cantharellales on roots after half year and one year of decay, suggesting that ectomycorrhizal fungi may become saprotrophic after root senescence. Further phylogenetic analysis is needed to identify the important fungal species that are involved in fine root decomposition, which will shed further light on the controls of the fine root decay process.

INTRODUCTION:

Root turnover and decomposition is a major pathway for carbon and nutrient flux from plants to soil (Parton and others 2007; Bird and others 2008). At the global scale, fine root turnover has been estimated to account for over one-third of net primary productivity (Jackson and others 1997). Inputs of organic matter from dead fine roots can result in more C stabilized in soil and more nutrients released than aboveground litter (Vogt and others 1986). Despite the importance of fine roots to soil C sequestration and nutrient cycling, relatively little attention has been paid to root decomposition, and few principles have been established on factors that regulate the root decay process (Berg 1984; Silver and Miya 2001).

In a review of root decomposition data, Silver and Miya (2001) suggested that root chemistry indices, such as C:N ratio and Ca concentration, and climate factors like actual evapotranspiration are the primary controllers on root decomposition. However, the possible roles of decay microorganisms and soil environment have not been thoroughly evaluated.

Roots form complex associations with rhizosphere microbes throughout their life time (Singh and others 2004). Root decay microorganisms are derived from both these pre-mortality

rhizosphere microbes and the wider soil microbial community (Fisk and others 2011). Recent studies have shown that the rhizosphere organisms have a strong impact on root decay patterns following root senescence. In fact, Langley and others (2006) suggested that fungal colonization may prevail over root chemistry in regulating fine root decomposition. In addition, studies of saprotrophic fungi on leaf litter suggest that an important component of the decomposition process is the formation of fungal hyphal networks, which transport organic C and mineral nutrients and thereby facilitate the utilization of heterogeneous soil organic matter substrates (Tlalka and others 2008). Hyphal networks could also play an important role in root decomposition because of the highly heterogeneous distribution of dead fine roots in soil.

The role of soil environment in root decay has also received little study. In northern forest ecosystems, fine roots are distributed in both organic and mineral soil horizons, which differ in C availability, nutrient status, moisture, faunal abundance, microbial community, and edaphic conditions (Parmelee and others 1993). Microbes living in surface organic horizons, which depend on labile C supply from leaf litter, are often N limited, while microbes living in mineral soils, which derive their C and energy from recalcitrant soil organic matter, are limited primarily by C (Fontaine and others 2003; Fontaine and Barot 2005). Thus, dead roots of the same individual tree are exposed to very different environments and decomposer groups. Also, fine roots in organic and mineral soil horizons differ significantly in organic matter quality and mineral nutrient concentrations (Fahey and others 1988). These differences in root chemistry, decomposers, and environment between organic and mineral soils may lead to distinct patterns of

fine root decomposition in the two soil horizons.

By far, most of our understanding of fine root decay comes from experiments using the litterbag approach (Silver and Miya 2001), yet the appropriateness of this method has been questioned (Fahey and others 1988; Fahey and Arthur 1994; Dornbush and others 2002). During litterbag preparation, roots are separated from soil, washed and dried, and the rhizosphere associations that roots developed over their lifetime are destroyed (Bloomfield and others 1993; Dornbush and others 2002). This may influence the microbial community development on decomposing fine roots (Fisk and others 2011) and result in an anomalously low decay rate (Dornbush and others 2002). In contrast, intact core technique, a method developed by Dornbush and others (2002), can largely avoid this problem by keeping normal rhizosphere conditions intact. Understanding the extent to which these two methods affect microbial decomposer communities and the root decomposition process is important in that it allows us to critically evaluate the results from past root decomposition studies using litterbags.

The aim of the present study was to: 1) evaluate the role of fungal hyphal networks in regulating root decomposer community and decay rates in organic and mineral horizons; 2) disentangle the interactive effects of substrate chemistry and soil environment on fine root decomposition; 3) evaluate the appropriateness of traditional root decay research with litterbags. To examine the response of fungal decomposer communities to different experimental approaches and treatments, we applied 454 pyrosequecing in our study, which is very effective in revealing the high fungal diversity in soil (Buee and others 2009). We hoped that results from this study would enhance

our understanding of the factors that regulate the fine root decomposition process in forest ecosystems, and provide insight on the design of future root decomposition studies.

METHODS:

Study area and field experiments:

To address our questions, we conducted two parallel experiments. Experiment 1 is an *in-situ* root decomposition experiment with intact cores, designed to examine the impact of filamentous fungal network on fine root decomposition. Experiment 2 is a reciprocal fine root transplant experiment with litterbags, designed to compare the effects of root chemistry and soil environment on the root decay process. With results from these two experiments, we can compare the intact core approach to the traditional litterbag approach in root decomposition studies.

Both experiments were conducted at the Pack Demonstration Forest of SUNY-ESF located near Warrensburg in the southeastern Adirondacks, New York, USA (43.55°N, 73.82°W). The Pack Plantations are situated on a highly uniform, sandy outwash plain, and include several replicate monospecific plots of red pine (Pinus resinosa Sol. ex Aiton; Nowak and others 1989). A thick organic horizon with abundant roots has developed on the surface of the mineral soil (Nowak and others 1991) and root distribution is relatively homogeneous because of the regular tree spacing and the uniform mineral substrate (Nowak, pers. commu.) These characteristics are crucial for root decomposition experiments with intact cores (experiment 1), which requires relatively low

spatial variation in fine root biomass (Dornbush and others 2002).

For experiment 1, we conducted a preliminary survey of fine root density in the field site before the incubation experiment. At May 6th, 2010, we collected twenty intact soil cores, 30 cm deep by 5cm in diameter, from a red pine monoculture using PVC corers. The soil cores were separated into organic and mineral horizons based on color and texture, stored in a cooler, and brought back to lab on the same day. Fine roots (< 1mm diameter) from each soil horizon in each soil core were picked out, and their masses and nutrient contents were determined. The average dry weight of fine roots in organic horizon and fine roots in mineral horizon among cores were 0.178g and 0.550g, respectively, and the coefficient of variation of dry weights among cores was stable at n = 15 cores. The average density of roots at 0-30 cm depth in our study site is 92.7 g/m^2.

On May 24th, 2010, sixty intact soil cores of the same size as in preliminary survey were collected at the same site with PVC pipes. Each of these PVC pipes had been drilled with holes on the walls so that a total of 40% of surface area of each pipe was holes. Each core was covered with 53-micrometer pore size mesh to allow the passage of fungal hyphae but not roots, and was implanted back into the ground at the collection location to start the incubation. The cores are arranged in pairs: in each pair, one core remained static during experiment period, and the other one was rotated every month to sever ingrowing fungal hyphae.

For experiment 2, we harvested roots from organic and mineral soil horizons at the same site on May 6^{th}, 2010 and brought them back to lab on the same day stored in a cooler. Fine roots were

picked out, gently washed free of soil, air dried, and a measured weight of dry roots was placed in 15 × 15 cm litterbags made of 53-micrometer pore size mesh. Each litterbag of fine roots from organic horizon was filled with 1.90 g dry roots, and each litterbag of fine roots from mineral horizon was filled with 1.85 g dry roots. On May 24th, 2010, litterbags were buried at six plots, chosen randomly at the study site. Each plot consisted of four subplots, with a distance of 30 cm from each other. In one subplot, one bag of organic soil fine roots was incubated in the organic horizon at 5 cm depth; in another subplot, one bag of organic soil fine roots was incubated in the mineral horizon at 15-20 cm depth. In the other two subplots, two bags of mineral soil fine roots were incubated in the same way in the organic horizon and mineral horizon, respectively.

Fifteen pairs of intact cores and twelve litterbags from three plots were chosen randomly and harvested at approximately 0.5 yr from decay initiation (Nov 10th, 2010), and the other half of samples were harvested at approximately one year from decay initiation (Jun 23rd, 2011). Once collected, soil cores were separated into organic horizon and mineral horizon, and separated soil cores and litterbags were immediately put in ziploc bags, placed on ice in a cooler, transported to the laboratory within a few hours, and frozen at -20°C for later processing. Soil cores were soaked in water to loosen the adhering soil particles. Fine roots were later picked and washed with dH_2O to remove soil.

Chemical Analysis:

Fine roots from each soil core and litterbag were oven-dried to constant weight at 55°C for 48 hrs, and weighed. For chemical analysis, root samples from each collection date were pooled by soil horizon, treatments (i.e. fine roots from static cores or rotated cores, and original or transplanted soil horizons for litterbag roots). Samples were ground into fine powder, and sent to DairyOne Laboratory (DairyOne Cooperative Inc) at Ithaca, NY, USA for total nitrogen, phosphorus and calcium analysis. Total N was analyzed by Leco FP-528 Nitrogen/Protein Analyzer, with an average coefficient of variation of analysis at 1.5 %. Total P was analyzed by Intrepid Inductively Coupled Plasma (ICP) Radial Spectrometer, with an average CV of analysis at 4%. All chemical indices are expressed on an ash-free, dry-mass basis.

Molecular biology analysis:

To examine the effect of experimental methods (litterbag or intact core), root type (organic soil roots or mineral soil roots), and treatments (i.e. fine roots from static cores or rotated cores, and original or transplanted soil horizons for litterbag roots) on fungal decomposer community of fine roots, we used parallel 454 pyrosequencing and DNA-tagging. From each fine root sample, total DNA was extracted and eluted using UltraClean Soil DNA Isolation Kit (MoBio Laboratories), following manufacturer's instructions. Templates were then quantified with a ND1000 spectrometer (NanoDrop Technologies). To produce amplicon libraries, we used fungal primer pairs ITS1f (5'- A–MID–CTTGGTCATTTAGAGGAAGTAA -3') (Gardes and Bruns, 1993) and ITS2 (5' B–MID–GCTGCGTTCTTCATCGATGC -3') (White 1990), where A

and B represent the two pyrosequencing primers (CGTATCGCCTCCCTCGCGCCATC-AG and CTATGCGCCTTGCCAGCCCGCTCAG), and MID were sample-specific barcodes, chosen from the recommended barcodes list in the technical bulletin of Genome Sequencer FLX System (454 Life Sciences, USA). MID (DNA tags) were used here for later allocating the sequences into the specific samples to which they belong. Each sample was PCR-amplified in 50 μL volumes in three separate reactions. Each reaction contained 10 ng of the template DNA, 200 nM of each primer, and 45 μL Invitrogen Platinum PCR SuperMix (Invitrogen Corporation, CA). The PCR conditions used were 94°C for 3 min, 25 cycles of 94°C for 1 min (denaturation), 54°C for 1 min (annealing), and 72°C for 2 min (extension), followed by 72 °C for 10 min (final extension). Together with normal PCR reactions, a negative control that contained all reagents but template was run for each sample. To check for possible amplification of contaminants, gel electrophoresis was performed for all PCR products. No sign of contamination was found. The three amplicons for each sample were combined and purified using QIAquick PCR Purification Kit (Qiagen Inc, USA). An equimolar mix of all sixteen amplicon libraries was sent for 454 pyrosequencing on the Genome Sequencer FLX 454 System (454 Life Sciences) at the Cornell University Life Science Core Laboratory Center.

Sequence editing and OTU designation:

For quality control, sequences without DNA tags or primer sequences as well as sequences that were shorter than 200 bp were removed. In sequences that passed quality control, DNA tags

and primer sequences were removed before OTU designation. We used mothur v1.25.1 (Schloss and others 2009) to assign OTUs. A FASTA file that contains only the unique sequences was created, together with a names file that shows the relationship between sequences that are identical. The distance matrix among unique sequences was calculated. Sequences were then clustered into OTUs based on the distance matrix file and names file. The distance cutoff for clustering was set to 0.01. According to the calculation method of distance in mothur, a 0.01 distance between two sequences approximately corresponds to 99% similarity between two sequences. For OTU clustering, the distance cutoff at 0.01 is reasonable given that the variation of the ITS1 region of fungal ribosomal DNA among morphologically distinct fungal species is high, but the intraspecific variation is low (Gardes and Bruns, 1993). On average, a 1-3% sequence dissimilarity in ITS region is appropriate for distinguishing fungal species, yet exceptions exist in which intraspecific variation is higher (Nilsson and others 2008). Sequences were parsed by sample to calculate the abundance (i.e. the number of reads) of all OTUs in each sample, resulting in an OTU by sample matrix. The most abundant fifty fungal OTUs were used as BLAST queries to search NCBI nonredundant nucleotide database on May 25th, 2012. The searches excluded uncultured and environmental sample sequences to identify the best BLAST matches, and their distribution (i.e. the number of reads) in four groups of samples were calculated: i.e. 0.5 yr intact core samples, 0.5 yr litterbag samples, one year intact core samples, one year litterbag samples. We present the NCBI closest matches only as tentative identifications and interpret them with caution.

Statistical Analysis:

We fitted the mass remaining % of fine roots in intact core and litterbag experiments to the exponential decay model (Mass remaining % = Ce^{kt}, t is time, k is the decay rate, C is a coefficient). We analyzed the differences in mass remaining of litterbag root samples among root types (organic soil fine root and mineral soil fine root), decay environments, and sample time with a mixed level (2×2×2) three-way factorial ANOVA. The percentage mass loss of root samples from different experiment treatments were compared by independent sample *t*-tests. We compared root decay rate between different root types or experiment treatments using a multivariate regression model that contains a dummy variable to distinguish data from different root type or treatment groups (dummy-variable regression model, Kleinbaum and others 1998). The model used was:

$$Y = \alpha + \beta X + \gamma Z + \delta XZ + \varepsilon \qquad \text{eqn 1}$$

where Y = log (mass remain %), X = time (yr), and Z was a dummy variable indicating the root type or treatment group to which the data belonged, with 0 representing one group, and 1 representing the other. As the model could be written as:

$$Z = 0 : Y = \alpha + \beta X + \varepsilon \qquad \text{eqn 2}$$

$$Z = 1 : Y = (\alpha + \gamma) + (\beta + \delta)X + \varepsilon \qquad \text{eqn 3}$$

the difference between decay rates can be examined by the significance of δ. We used linear regression to estimate model parameters (Eqn 1) and their significance. When δ was significant,

the regression slopes (i.e. the decay rates) for the two groups were significantly different (Eqns 2, 3). These analyses were performed using SPSS software (2001, ver. 13.0, SPSS Inc., USA). For the fungal community analysis, fungal diversity indices were calculated for the OTU by sample matrix using R software v. 2.13, and rarefaction analysis was performed with ANALYTIC RAREFACTION v.1.4. (Hunt Mountain Software, Department of Geology, University of Georgia, Athens, GA, USA). To visualize the fungal community data, in each sample we calculated the frequency of different OTUs, and applied Non-metric Multidimensional Scaling (NMDS) multivariate analysis on the frequency data using R software. The two dimensional solutions were selected, with the pairwise community distances estimated by Bray-Curtis index.

RESULTS:

The average initial dry mass of organic soil fine roots and mineral soil fine roots in intact cores were 0.178 g (standard error ± 0.039 g) and 0.550 g (standard error ± 0.043 g), respectively (Table 2.1). Not surprisingly, initial tissue chemistry was different between organic horizon and mineral horizon fine roots, with much higher concentrations of N, P, and Ca in organic soil fine roots (Table 2.1).

After one year's decomposition, the fraction of fine root mass loss was significantly greater within intact cores than within litterbags ($P < 0.001$). On average, organic soil fine roots lost 23.6 % more weight within intact cores than within litterbags, and mineral soil fine roots lost

Table 2.1 Initial tissue chemistry for red pine fine roots in each intact core and litterbag, categorized by the soil horizon that roots come from. Samples were collected from Pack Forest, New York.

Treatment	N (%)	P (%)	Ca (%)	C (%)	Lignin (%)
Organic soil fine roots	1.3	0.22	0.5	51.0	45.1
Mineral soil fine roots	0.9	0.15	0.31	45.8	32.7

33.3 % more weight within intact cores than within litterbags (Figure 2.1). For both organic soil and mineral soil fine roots, mass loss during the first year was not significantly different between roots within rotated soil cores and static cores ($P = 0.604$ for organic soil fine roots; $P = 0.330$ for mineral soil fine roots; Table 2.2). First year mass loss was also not significantly different between litterbag roots placed at organic soil and mineral soil horizons ($P = 0.540$ for organic soil fine roots; $P = 0.518$ for mineral soil fine roots). In intact cores, though the decay rates (k value) of mineral soil fine roots tended to be greater than for organic soil fine roots, they were not significantly different (differences in decay rate was indicated by the significance of δ in dummy variable regression test; here $P = 0.138$ for δ; Table 2.2), and their first year mass loss was not significantly different. The great variation of root mass among soil cores precluded the detection of these smaller effects. In litterbags, organic soil fine roots decomposed faster than mineral soil fine roots, and the first year mass loss of organic soil roots was significantly greater than that of mineral soil roots ($P = 0.015$; Table 2.3).

Throughout decomposition, the variation of fine root biomass remaining among samples in each litterbag treatment was very low (coefficient of variation, CV <5.4%), while the variation among samples in each intact core treatment was high, especially among organic soil samples. During the first year, the CV among organic soil fine roots in intact cores declined from 97% (at time zero) to 60 – 70%, and the CV among mineral soil fine roots in intact cores remained around 35 – 40%.

In both rotated and static soil cores, organic soil fine roots and mineral soil fine roots lost about

Figure 2.1 Trend of mass and N, P contents during root decomposition in different experiment treatments, based on comparison with original levels. (A) organic soil roots in soil cores, (B) mineral soil roots in soil cores, (C) organic soil roots in litterbags, (D) mineral soil roots in litterbags. OS-R, OS-S represents organic soil roots decomposing in rotated soil cores and in static soil cores, respectively. MS-R, MS-S represents mineral soil roots decomposing in rotated soil cores and in static soil cores, respectively. (OS) OS, (OS) MS represents organic soil roots decomposing in litterbags placed at organic soil and mineral soil, respectively. (MS) OS, (MS) MS represents mineral soil roots decomposing in litterbags placed at organic soil and mineral soil, respectively. Error bars denote standard errors.

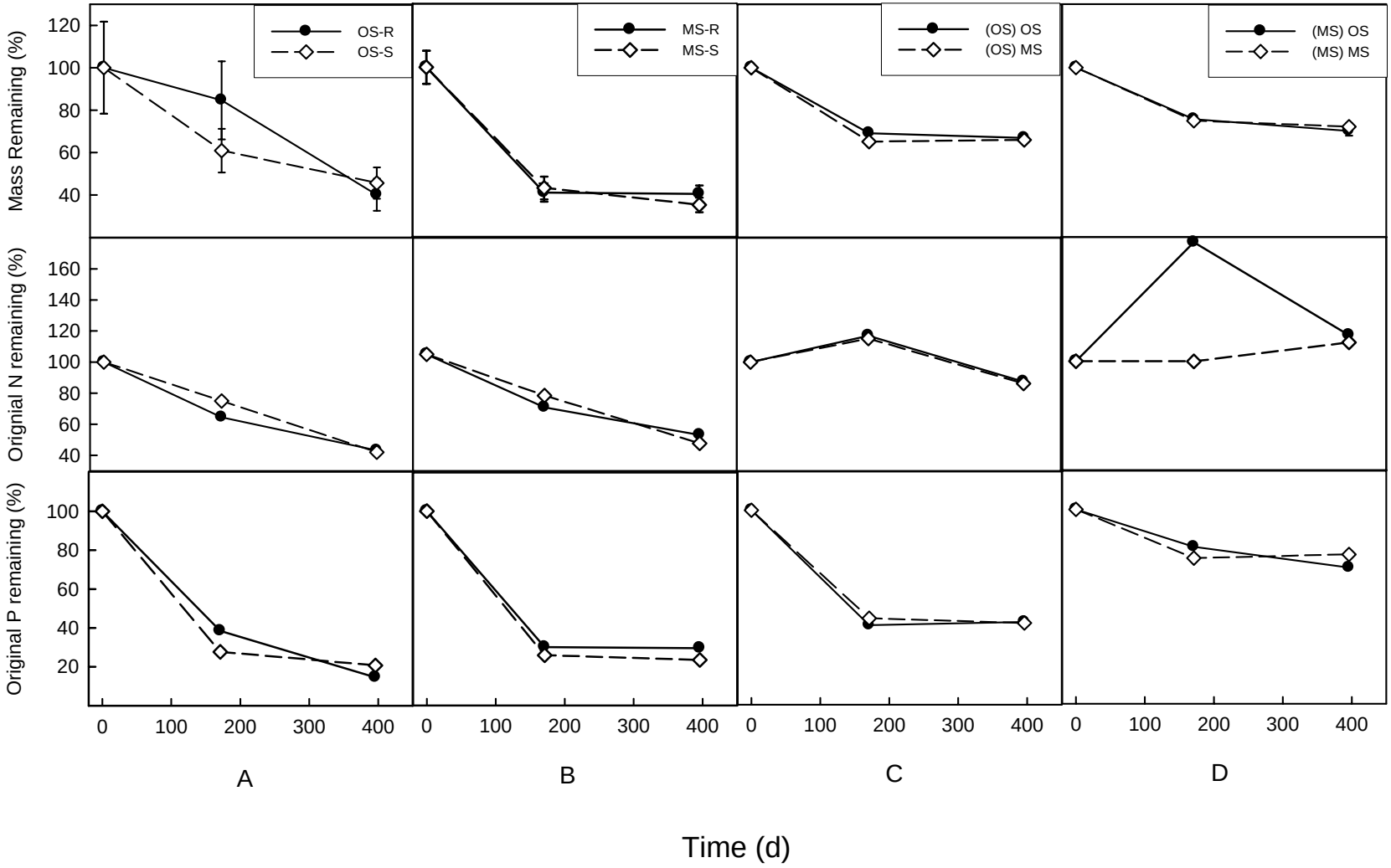

Mass Remaining (%)
Orignial N remaining (%)
Original P remaining (%)
OS-R
OS-S
MS-R
MS-S
(OS) OS
(OS) MS
(MS) OS
(MS) MS
A
B
C
D
Time (d)

Table 2.2 Exponential decay curve fits of fine root mass remaining % in intact core and litterbag experiments (Mass Remaining % = Ce^{kt}). Coef denotes the C value in the formula. Std. Errors and P values denote the standard error and the significance of k value, respectively. OS and MS are abbreviations for organic soil and mineral soil, respectively.

Method	Original soil horizon	Treatment	Coef	k value	Std. Error	P value	R^2
Intact Core	Organic soil fine roots	Rotated cores	1.027	−0.7389	0.374	0.054	0.113
		Static cores	0.985	−0.787	0.379	0.044	0.116
	Mineral soil fine roots	Rotated cores	0.968	−1.092	0.199	<0.001	0.509
		Static cores	0.978	−1.181	0.207	<0.001	0.562
Litterbag	Organic soil fine roots	Decay in OS	0.954	−0.406	0.081	0.002	0.784
		Decay in MS	0.944	−0.431	0.103	0.004	0.717
	Mineral soil fine roots	Decay in OS	0.968	−0.345	0.057	0.001	0.842
		Decay in MS	0.962	−0.320	0.063	0.002	0.785

Table 2.3 Results of mixed-level three-way factorial ANOVA on the mass remaining of fine root samples in litterbags for red pine root decay study at Pack Forest, New York.

ANOVA			
Source of variation	df	*P* values	F values
Root type	1	**0.000**	**21.965**
Decay environment	1	0.354	0.913
Sample time	1	**0.024**	**6.263**
Root type × Decay environment	1	0.131	2.536
Root type × Sample time	1	0.095	3.149
Decay environment × Sample time	1	0.153	2.255
Root type × Decay environment × Sample time	1	0.893	0.019

55-61% of total N following one year's decomposition (Figure 2.1). In all litterbag samples, N was immobilized in the first 0.5 yr of decay. After one year, organic soil fine roots in litterbags lost about 12-14 % of total N, and mineral soil fine roots in litterbags gained about 12-17% N (Figure 2.1). P loss rate also was much higher from roots decaying in intact cores than from roots decaying in litterbags, and was higher from organic soil fine roots than from mineral soil fine roots. In one year, 79-85% of original P was lost in organic soil fine roots in intact cores, and 70-77% in mineral soil fine roots in intact cores, whereas 57-58% of original P was lost in organic soil fine roots in litterbags, and 23-30% in mineral soil fine roots.

Among all sequence outputs of 454 pyrosequencing analysis, a total of 237627 sequences passed the quality control. The average length of qualified ITS-1 sequences was 267 bp. The number of reads per sample ranged from 5000 to 28934, with an average of 14852 per sample. The acquired sequences represented a total of 13907 unique OTUs at the distance cutoff of 0.01 among sequences in mothur clustering, among which 3750 OTUs were non-singletons (see Methods for explanations). The number of OTUs identified in each sample ranged from 161 to 2662, with an average of 1266 per sample. The number of sequences, OTUs, and singletons in each sample, and the Chaos 1 estimator of each sample are presented in Table S 2.1. The Fisher's α diversity index, Simpson's diversity, Shannon's diversity, and evenness of fungal communities of each sample are presented in Table S 2.2.

We used two dimensional NMS to visualize fungal communities on decaying roots of different treatments and decomposition periods. NMS analysis was conducted on the OTU frequency

Table S 2.1 The number of total sequences, OTUs, singletons, and the Chao 1 estimator (and its 95% confidence interval) of fungal communities in samples collected in Nov 2010 and Jun 2011. Chao 1 estimator is the estimated total species richness of a sample. OS-R, OS-S represents organic soil roots decomposing in rotated soil cores and in static soil cores, respectively. MS-R, MS-S represents mineral soil roots decomposing in rotated soil cores and in static soil cores, respectively. (OS) OS, (OS) MS represents organic soil roots decomposing in litterbags placed at organic soil and mineral soil, respectively. (MS) OS, (MS) MS represents mineral soil roots decomposing in litterbags placed at organic soil and mineral soil, respectively.

Sample	# sequences	# OTUs	# singletons	Chao 1 (95% C.I.)
Nov 2010 samples				
OS-R	15968	1283	875	4227.7 (3647.7 – 4949.9)
OS-S	12859	1154	773	3643.7 (3131.4 – 4288.7)
MS-R	9163	813	514	2280.8 (1930.2 – 2741.3)
MS-S	15372	1393	987	4799.2 (4159.2 – 5587.3)
(OS) OS	16523	1110	740	3138.1 (2729.0 – 3650.8)
(OS) MS	13169	1031	689	2763.6 (2409.7 – 3208.3)
(MS) OS	13164	997	649	2668.4 (2316.1 – 3114.9)
(MS) MS	12750	929	615	2696.4 (2305.3 – 3198.6)
Jun 2011 samples				
OS-R	5000	597	370	1463.5 (1235.2 – 1773.3)
OS-S	11955	1259	861	4177.6 (3597.5 – 4901.5)
MS-R	13942	1407	968	4372.3 (3827.2 – 5040.1)
MS-S	28934	2366	1723	8819.8 (7856.5 – 9952.0)
(OS) OS	26723	2662	1912	9508.0(8546.2 – 10626.9)
(OS) MS	16788	1634	1160	5761.6 (5034.4 – 6644.3)
(MS) OS	6078	161	104	377.3 (285.3 – 537.3)
(MS) MS	18609	1462	1052	4986.5 (4348.0 – 5766.4)

Table S 2.2 Fisher's α diversity index, Simpson's diversity, Shannon's diversity, and Evenness of fungal communities in samples collected in Nov 2010 and Jun 2011. For each sample, Evenness is calculated as the ratio Shannon's diversity / ln (Richness). OS-R, OS-S, MS-R, MS-S, (OS) OS, (OS) MS, (MS) OS, (MS) MS mean the same as in table S 2.1.

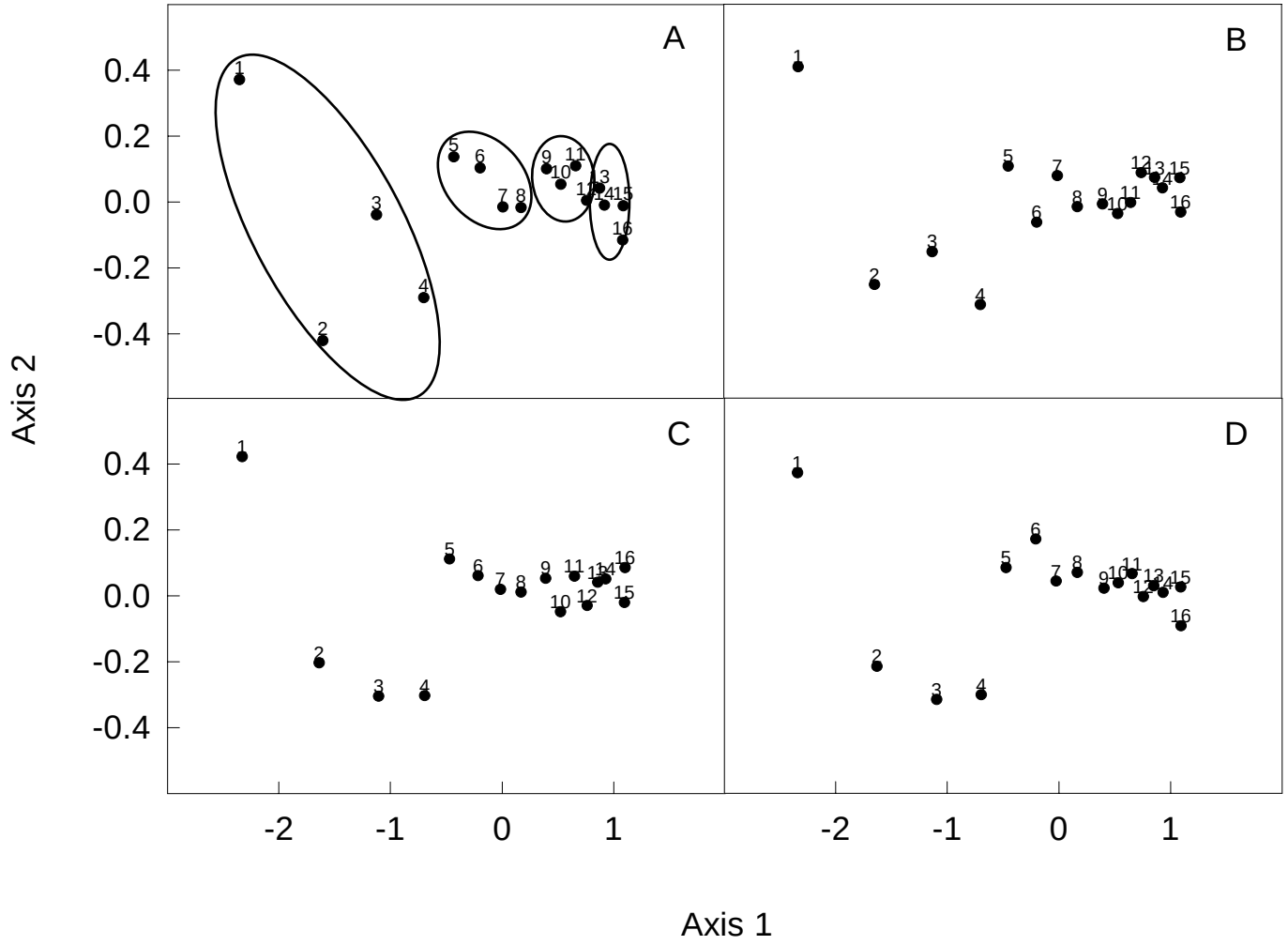
Sample	Fisher's α	Simpson's diversity	Shannon's diversity	Evenness
Nov 2010 samples				
OS-R	328.66	0.95	4.28	0.60
OS-S	307.05	0.96	4.55	0.65
MS-R	215.45	0.96	4.28	0.64
MS-S	371.91	0.96	4.46	0.62
(OS) OS	268.36	0.89	3.57	0.51
(OS) MS	261.84	0.95	4.04	0.58
(MS) OS	250.45	0.93	3.99	0.58
(MS) MS	230.46	0.94	3.88	0.57
Jun 2011 samples				
OS-R	176.78	0.97	4.43	0.69
OS-S	355.06	0.97	4.72	0.66
MS-R	390.53	0.98	4.76	0.66
MS-S	609.69	0.95	4.43	0.57
(OS) OS	735.34	0.98	5.22	0.66
(OS) MS	447.56	0.98	4.91	0.66
(MS) OS	n.a.	0.59	1.31	0.26
(MS) MS	371.72	0.94	4.14	0.57

matrices that included 1) all OTUs, 2) all OTUs except singletons, 3) OTUs that had more than 10 associated sequences, and 4) the most abundant one hundred OTUs among all samples (Figure 2.2). For all NMS analyses, the stress levels (i.e. badness of fit) were under 0.02, showing a high NMS model quality. The patterns of the NMS analyses on different frequency datasets were almost identical. The first axis clearly separates fungal communities on roots after 0.5 yr decomposition from those on roots after one year's decomposition (Figure 2.2). In samples from each of the collection dates, fungal communities on intact core roots were also clearly separated from fungal communities on litterbag roots (Figure 2.2). The differences among fungal communities on roots after 0.5 yr decay were larger than after one year's decay (Figure 2.2).

For a closer look at the distribution of fungal taxa across samples, we combined the fungal community data of the sixteen samples into four groups – 0.5 yr intact core samples, 0.5 yr litterbag samples, one year intact core samples, and one year litterbag samples; this procedure was justified by the relative similarity of fungal community composition among samples in these groups, shown by NMS (Figure 2.2). We present the closest NCBI database match of the most abundant fifty fungal OTUs among all samples in Table S 2.3. These fifty OTUs are listed in descending order according to their abundance in fungal communities on 0.5 yr intact core roots, for better visualization and comparison of the distributions of these OTUs among fungal communities of the four sample groups.

There was a clear transition in fungal community composition from 0.5 yr root samples to one

Figure 2.2 Non-metric Multidimensional Scaling (NMS) of fungal communities on decaying fine roots from the sixteen samples in our study. (A)(B)(C)(D) are NMS on the OTU frequency matrix that include (A) all OTUs, (B) all OTUs except singletons, (C) OTUs that had more than 10 associated sequences, and (D) the most abundant one hundred OTUs among all samples. Number 1-8 denote 0.5 yr samples: 1-organic soil fine roots in rotated cores, 2-organic soil fine roots in static cores, 3-mineral soil fine roots in rotated cores, 4-mineral soil fine roots in static cores, 5-organic soil fine roots in litterbags placed at organic soil, 6-organic soil fine roots in litterbags placed at mineral soil, 7-mineral soil fine roots in litterbags placed at organic soil, 8-mineral soil fine roots in litterbags placed at mineral soil. Number 9-16 denote one year samples in the same order as for 0.5 yr samples. In (A) we encircled 1-4 (0.5 yr intact core samples), 5-8 (0.5 yr litterbag samples), 9-12 (one year intact core samples), 13-16 (one year litterbag samples) to show the similarity of fungal communities in each group and the dissimilarity of fungal communities among groups.


A
B
C
D
Axis 2
Axis 1
0.4
0.2
0.0
-0.2
-0.4
-2
-1
0
1

year root samples (Table S 2.3). Together, the fifty most abundant fungal OTUs represented 61.8% of the total reads. The most frequent OTU was assigned to *Phialocephala fortinii* complex, which is a dark septate tree-root endophyte (Grunig and others 2004). The most abundant fifty OTUs were distributed in three phyla and eleven orders – Ascomycota (Helotiales, Archaeorhizomycetales, Peltigerales), Zygomycota (Mortierellales), Basidiomycota (Filobasidiales, Boletales, Agaricales, Thelephorales, Cantharellales, Atheliales, Hymenochaetales) (Table 2.4, S2.3).

By combining these fifty OTUs by taxonomic order, we obtained the fungal community compositions in 0.5 yr intact core samples, 0.5 yr litterbag samples, one year intact core samples, and one year litterbag samples on the order level (Table 2.4). Among the eleven orders, Helotiales (saprotrophic ascomycetes) was relatively evenly distributed among all four groups, while the zygomycetes, Mortierellales and Mucoromycotina, were mostly found in root samples after 0.5 yr of decomposition. The Archaeorhizomycetales, Agaricales, Hymenochaetales, and Thelephorales were mostly found in root samples after one year's decomposition. Filobasidiales and Boletales were concentrated in 0.5 yr intact core root samples, whereas Atheliales were concentrated in 0.5 yr litterbag samples, and Cantharellales were concentrated in one yr intact core samples. On the phylum level, there was also a distinct distribution of fungal phyla among four groups (Table 2.5). In 0.5 yr intact core samples and 0.5 yr litterbag samples, Zygomycota accounted for 28.9% and 53.4% of the reads among the most abundant fifty OTUs, whereas in one year intact core samples and litterbag samples, it only accounted for 0.25% and

Table S 2.3 NCBI database match of the fifty most abundant fungal OTUs in our study. The number of reads of these OTUs in four groups – 0.5 yr intact core samples, 0.5 yr litterbag samples, one year intact core samples, and one year litterbag samples are listed, with the numbers over 1000 in bold.

Closest NCBI database match	NCBI accession #	Max/ Total score	Query coverage/ Max identity	E value	0.5 yr intact core root samples	0.5 yr litterbag root samples	One year intact core root samples	One year litterbag root samples	Total Reads
Phialocephala fortinii complex	HM190136.1	483/483	99 % / 99 %	3e-133	**6262**	**7378**	**1835**	**2744**	18219
Mortierella	AB542098.1	158/158	39 % / 94 %	2e-35	**3583**	**1695**	5	3	5286
Cryptococcus	FN298664.1	405/405	92 % / 99 %	7e-110	**2520**	842	1	4	3367
Mortierella	JF439486.1	418/418	100% / 99%	8e-114	**2380**	**9105**	40	136	11661
Helotiaceae	HQ157877.1	416/416	86 % / 97 %	4e-113	**1770**	631	662	888	3951
Xerocomus	HM190050.1	436/436	100%/ 98%	2e-119	**1338**	1	244	58	1641
Unknown ascomycota	FJ008693.1	340/340	94 % / 95 %	2e-90	**1294**	**1217**	460	**1231**	4202
Mortierella	EU918703.1	176/176	100% / 80%	6e-41	**1293**	529	8	8	1838
Scleroderma	HM189957.1	470/470	100%/99%	3e-129	**1286**	33	176	0	1495
Unknown mucoromycotina	JF340267.1	226/226	100%/85%	5e-56	**1209**	380	1	4	1594
Hydropus	DQ490627.1	571 / 571	82 % / 98 %	1e-159	**1192**	0	6	57	1255
Phialocephala fortinii	HM190136.1	468 / 468	98 % / 99 %	9e-129	**1179**	956	885	538	3558

Table S 2.3 (Continued)

Mortierella	AJ878782.1	381 / 381	93% / 98%	1e-102	870	**2410**	5	9	3294
Pseudotomentella	AJ889968.1	527/527	100%/99%	2e-146	733	0	534	0	1267
Mortierella	EU240039.1	396 / 396	100 % / 99 %	4e-107	670	**2116**	2	9	2797
Pseudotomentella	AJ889968.1	505/505	100% / 98%	8e-140	664	1	**1038**	0	1703
Phialocephala fortinii complex	EU882733.1	484 / 484	100 % / 99 %	9e-134	635	520	255	48	1458
Pseudotomentella	AJ889968.1	339/339	100% / 88%	8e-90	498	96	**2023**	**1493**	4110
Unknown zygomycetes	AM292198.1	298/298	99%/90%	1e-77	472	626	2	8	1108
Unknown ascomycota	FJ008693.1	353/353	93%/96%	2e-94	472	192	66	262	992
Unknown mucoromycotina	JF340267.1	195 / 195	100 % / 83 %	2e-46	469	**1632**	1	0	2102
Phialocephala fortinii complex	HM190136.1	444 / 444	100 % / 99 %	1e-121	411	926	232	506	2075
Clitocybula	JF730328.1	257/257	100% / 82%	3e-65	400	154	353	24	931
Clavulina	JN228228.1	483/483	100%/97%	4e-133	299	563	780	268	1910
Unknown ascomycota	FJ008693.1	350/350	94%/95%	3e-93	179	432	202	263	1076
Pseudotomentella	AJ889968.1	361/361	100%/88%	2e-96	170	37	913	874	1994
Unknown zygomycetes	AM292198.1	359/359	99%/96%	5e-96	117	910	9	14	1050

Table S 2.3 (Continued)

Amphinema	JN943915.1	292/292	100%/87%	6e-76	111	710	57	67	945
Henningsomyces	AY571044.1	272/272	99%/83%	1e-69	57	422	142	778	1399
Amphinema	AY838271.1	274/274	98%/86%	2e-70	56	831	39	192	1118
Leucopaxillus	EU819413.1	333/333	100%/87%	4e-88	35	619	**1924**	**2622**	5200
Mortierella	HQ608015.1	411/411	100%/99%	1e-111	35	947	4	267	1253
Mortierella	EF031110.1	453/453	89%/99%	3e-124	30	**1916**	2	3	1951
Mortierella	JF439485.1	409/409	99%/97%	5e-111	30	**1065**	12	17	1124
Leucopaxillus	EU819413.1	346/346	98%/87%	5e-92	29	2	**1788**	11	1830
Resinicium	DQ826537.1	427/427	98%/99%	1e-116	8	40	472	**2449**	2969
Leucopaxillus	EU819413.1	340/340	98%/87%	3e-90	0	0	**4835**	**4317**	9152
Pseudotomentella	AJ889968.1	340/340	98%/88%	2e-90	0	0	**3942**	**2693**	6635
Phialocephala fortinii complex	HM190136.1	446/446	98%/98%	4e-122	0	0	**2506**	**3815**	6321
Resinicium	DQ826537.1	422/422	97%/98%	7e-115	0	0	666	**3545**	4211
Archaeorhizomyces	JQ912673.1	198/198	97%/86%	1e-47	0	0	**1391**	**1295**	2686
Unknown ascomycota	FJ008693.1	340/340	91%/94%	2e-90	0	0	646	**1700**	2346

Table S 2.3 (Continued)

Helotiaceae	HQ157925.1	414/414	92%/96%	1e-112	0	0	871	**1077**	1948
Phialocephala fortinii complex	HM190136.1	479/479	98%/98%	4e-132	0	0	**1018**	772	1790
Phialocephala fortinii complex	EU882733.1	448/448	98%/98%	1e-122	0	0	894	833	1727
Clavulina	JN228228.1	492/492	97%/98%	6e-136	0	0	**1372**	341	1713
Henningsomyces	AY571044.1	278/278	96%/83%	2e-71	0	0	178	**1093**	1271
Archaeorhizomyces	JQ912673.1	191/191	97%/85%	2e-45	0	0	623	576	1199
Pseudotomentella	AJ889968.1	520/520	98%/98%	3e-144	0	0	**1169**	2	1171
Nephroma	DQ320562.1	132/132	28%/93%	2e-27	0	0	917	0	917

Table 2.4 Taxonomic distribution of the fifty most abundant fungal OTUs on the order level. The most abundant fifty OTUs are from eleven orders, and four phyla/subdivisions – Ascomycota, Zygomycota, Basidiomycota, and Mucoromycotina. The number of reads of each order in the four groups – 0.5 yr intact core samples, 0.5 yr litterbag samples, one year intact core samples, and one year litterbag samples are listed. In each group, the average number and standard error of reads among four samples are listed in the brackets. The average number and standard error of reads of each order among 16 samples are listed in the brackets under “Total Reads”.

Closest NCBI database match	Life History	0.5 yr intact core root samples	0.5 yr litterbag root samples	One year intact core root samples	One year litterbag root samples	Total Reads
Ascomycota:						
Helotiales	Saprotrophic	10257 (2564.25; 429.32)	10411 (2602.75; 196.67)	9158 (2289.5; 355.38)	11221 (2805.25; 1053.92)	41047 (2565.44; 274.32)
Archaeorhizomycetales	Unknown / saprotrophic	0 (0; 0)	0 (0; 0)	2014 (503.5; 98.87)	1871 (467.75; 252.51)	3885 (242.81; 87.28)
Peltigerales	Lichen forming fungi	0 (0; 0)	0 (0; 0)	917 (229.25; 141.82)	0 (0; 0)	917 (57.31; 40.77)
Unknown ascomycota.		1945 (486.25; 88.12)	1841 (460.25; 88.76)	1374 (343.5; 68.63)	3456 (864; 328.66)	8616 (538.5; 94.69)
Zygomycota:						
Mortierellales	Saprotrophic	8891 (2222.75; 441.59)	19783 (4945.75; 513.09)	78 (19.5; 5.81)	452 (113; 59.52)	29204 (1825.25; 539.65)
Unknown zygomycetes		589 (147.25; 42.26)	1536 (384; 151.02)	11 (2.75; 2.1)	22 (5.5; 3.84)	2158 (134.88; 53.27)
Basidiomycota:						
Filobasidiales	Saprotrophic	2520 (630; 83.59)	842 (210.5; 107.83)	1 (0.25; 0.25)	4 (1; 0.41)	3367 (210.44; 73.02)

Table 2.4 (Continued)

Boletales	Ectomycorrhizal	2624 (656; 279.81)	34 (8.5; 7.5)	420 (105; 36.42)	58 (14.5; 14.5)	3136 (196; 93.78)
Agaricales	Ectomycorrhizal/ saprotrophic	1713 (428.25; 298.74)	1197 (299.25; 191.21)	9226 (2306.5; 2177.4)	8902 (2225.5; 1389.17)	21038 (1314.88; 632.72)
Thelephorales	Ectomycorrhizal	2065 (516.25; 157.02)	134 (33.5; 13.02)	9619 (2404.75; 1127.12)	5062 (1265.5; 704.67)	16880 (1055; 378.05)
Cantharellales	Ectomycorrhizal	299 (74.75; 53.31)	563 (140.75; 74.30)	2152 (538; 197.77)	609 (152.25; 62.12)	3623 (226.44; 69.15)
Atheliales	Saprotrophic	167 (41.75; 21.06)	1541 (385.25; 149.05)	96 (24; 10.78)	259 (64.75; 37.23)	2063 (128.94; 51.79)
Hymenochaetales	Saprotrophic	8 (2; 1.68)	40 (10; 10)	1138 (284.5; 283.83)	5994 (1498.5; 1498.17)	7180 (448.75; 376.31)
Unknown mucoromycotina	Saprotrophic / plant pathogenic	1678 (419.5; 252.35)	2012 (503; 382.27)	2 (0.5; 0.29)	4 (1; 0.71)	3696 (231; 118.67)

Table 2.5 Taxonomic distribution of the fifty most abundant fungal OTUs on the phylum level. The number of reads in each phylum in the four groups – 0.5 yr intact core samples, 0.5 yr litterbag samples, one year intact core samples, and one year litterbag samples are listed. In each group, the average number and standard error of reads among samples were listed in the brackets. The average number and standard error of reads of each phylum among 16 samples are listed in the brackets under "Total Reads".

Closest NCBI database match	0.5 yr intact core root samples	0.5 yr litterbag root samples	One year intact core root samples	One year litterbag root samples	Total Reads
Ascomycota	12202 (3050.5; 503.02)	12252 (3063; 280.10)	13463 (3365.75; 400.19)	16548 (4137; 1409.96)	54465 (3404.06; 370.11)
Zygomycota	9480 (2370; 462.61)	21319 (5329.75; 541.34)	89 (22.25; 7.73)	474 (118.5; 59.14)	31362 (1960.13;580.25)
Basidiomycota	9396 (2349; 400.93)	4351 (1087.75; 208.32)	22652 (5663; 3222.33)	20888 (5222; 939.40)	57287 (3580.44;905.28)
Mucoromycotina	1678 (419.5; 252.35)	2012 (503; 382.27)	2 (0.5; 0.29)	4 (1; 0.71)	3696 (231; 118.67)

1.25% of these reads. Conversely, in the one year intact core samples and litterbag samples a much higher percentage of Basidiomycota reads were observed among the most abundant fifty OTUs (62.6% and 55.1%, respectively) compared with the 0.5 yr samples (28.7% and 10.9%, respectively).

DISCUSSION:

Both organic soil fine roots and mineral soil fine roots of red pine decomposed much faster in intact cores than in litterbags (Table 2.2). Similar results have been found in a previous experiment by Dornbush and others (2002), which compared the effect of intact core and litterbag approaches on fine root decomposition of fine roots of trees and grasses.

No effect of core rotation to sever fungal hyphae on the mass loss and nutrient release of fine roots was observed in our study for both organic soil and mineral soil fine roots (Figure 2.1). Although there is some uncertainty about this effect for organic soil fine roots because of the high variation among cores, this result is highly conclusive for mineral soil fine roots. Langley and others (2006) conducted a similar core rotation experiment to examine the influence of hyphal ingrowth on pine fine root decomposition, and they also found no significant difference between roots in rotated and static cores on mass loss and nutrient release over two years of decomposition. However, in our study, some differences between the fungal decomposer communities on rotated core root samples and static core root samples were noted, though these differences were not as large as those between fungal communities on root samples after half

year and one year of decomposition (Figure 2.2).

There was a significant effect of root type (from organic vs mineral soil horizon), yet no effect of soil environment on the mass loss and nutrient release of fine roots in litterbags, within one year of decomposition (Figure 2.1; Table 2.3). In litterbag experiment, the first year mass loss of organic soil fine roots was significantly greater than that of mineral soil fine roots ($P = 0.015$). Also, organic soil fine roots in litterbags lost N and P much faster than mineral soil fine roots in litterbags (Figure 2.1).

Dornbush and others (2002) suggested that the traditional litterbag approach in root decomposition studies underestimated the nutrient release rates from decaying fine roots. Results of our study also support this finding (Figure 2.1). For both organic soil and mineral soil fine roots in intact cores, total N was continuously released throughout decomposition, whereas for fine roots in litterbags, N was immobilized in the initial 0.5 yr of decomposition, then slowly released (Figure 2.1). Also, P loss was much faster for fine roots decaying in intact cores that fine roots in litterbags (Figure 2.1).

454 pyrosequencing revealed a clear succession of fungal communities for both intact core and litterbag samples from 0.5 through one year of decay (Figure 2.2; Table 2.4, 2.5). Among the most abundant fifty OTUs, a much higher percentage of Zygomycota reads and much lower Basidiomycota reads were observed after 0.5 yr than one yr for both intact core and litterbag samples (Table 2.5). The fact that Basidiomycota becomes more abundant after one year of decomposition may reflect the fact that many Basidiomycota are important agents of wood and

litter decay, and are capable of decomposing recalcitrant materials. Interestingly, among the seven Basidiomycota orders that are found in the most abundant fifty OTUs, Boletales, Thelephorales, and Cantharellales are typically ectomycorrhizal fungi. While Boletales reads were most common in 0.5 yr intact core root samples, Thelephorales, and Cantharellales prevailed in both intact core and litterbag root samples after one year. This suggests that some ectomycorrhizal fungi may have saprotrophic capacity, becoming important decomposers after root senescence.

We found a significant contrast of fungal communities on litterbag roots from those on intact core roots, after both 0.5 and one year of decay (Figure 2.2; Table 2.4, 2.5). We propose that this is due to the process of litterbag preparation, in which the rhizosphere associations of roots were destroyed and the early development of the microbial community on decomposing fine roots was altered (Fisk and others 2011). The difference between fungal communities on litterbag roots and those on intact core roots were reduced after one year of decomposition, but some contrasts persisted (Figure 2.2). It is unknown whether the differences of fungal communities between intact core root samples and litterbag root samples explains the differences in mass loss and nutrient release rate of samples between these two experimental approaches, but the large differences are suggestive of such effects. Future studies are needed to illustrate the relationship between the composition of fungal decomposer communities and the decomposition process of fine roots.

The information we obtained from 454 pyrosequencing revealed an ultra-high richness of fungal

species on decomposing fine roots, and allowed us to examine the effect of time, treatments and experimental approaches on fungal communities on decaying fine roots. Further work is needed on phylogenetic analysis of 454 pyrosequencing outputs. Past studies have suggested that ITS-1 sequences with an average length of c. 250 bp are sufficiently polymorphic to allow phylogenetic identification of fungal OTUs at the species or genus level (Buee and others 2009; Liu and others 2008; Nilsson and others 2009). Additional analysis based on phylogenetic information will allow us to better understand the fungal species that are involved in fine root decomposition process, and shed light on the factors that regulate the fine root decomposition process in forest ecosystems.

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